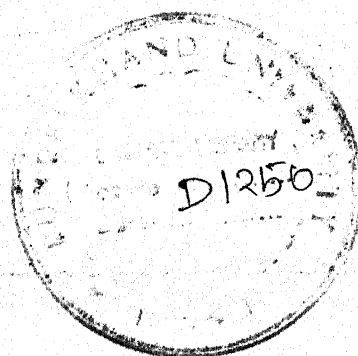


*Seroepidemiology of
hepatitis A, B, C, E in children
suffering from hepatitis
in Bundelkhand region*

**THESIS
FOR
DOCTORATE OF MEDICINE
[PAEDIATRICS]**



**BUNDELKHAND UNIVERSITY
JHANSI (U.P.)**

2004

S. Arif Ali Subzwari

*Dedicated to
my inspiration,
My Wife*

DEPARTMENT OF PAEDIATRICS

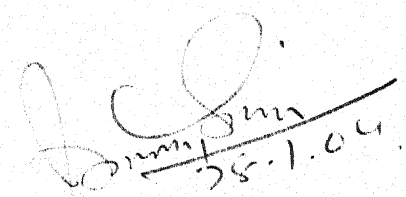
M.L.B. Medical College, Jhansi (U.P.)

CERTIFICATE

This is to certify that the work entitled
**"SEROEPIDEMIOLOGY OF HEPATITIS A,B,C,E IN CHILDREN
SUFFERING FROM HEPATITIS IN BUNDELKHAND REGION"**
has been carried by Dr. S.Arif Ali Subzwari in the Department
of Pediatrics, M.L.B. Medical College, Jhansi.

He has put necessary stay in the Department as per
University regulations.

Dated : 28th Jan '2003


Dr. (Mrs.) Sheela Longia
M.D.

Professor & Head,
Department of Pediatrics,
M.L.B. Medical College,
Jhansi (U.P.)

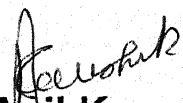
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which is being submitted as thesis for MD (Paediatric)
Examination 2004 has been carried out by Dr. S.Arif Ali
Subzwari himself under my direct supervision and guidance. The
observations recorded were checked and verified by me from
time to time.

Dated : 28th Jan '2009


Dr. Anil Kaushik
M.D.
Professor,
Department of Pediatrics,
M.L.B. Medical College,
Jhansi (U.P.)
(GUIDE)

DEPARTMENT OF PAEDIATRICS
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Dated : 28th Jan 2005


(Dr. R.K. Agrawal)

M.D.

Professor & Head,
Department of Microbiology,
M.L.B. Medical College,
Jhansi (U.P.)
(Co-GUIDE)

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As far as the achievements of a person are concerned they are a continuum in that he continuously is in search of new heights in his life, my self being no exception have reached this present moment in my life when I have to ponder over the persons who have mattered in my life and on account of whom I am what I am today.

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Behind every successful man there is a woman, I always thought that this was just a quote, but she proved me wrong, she is my wife Nikhat who has been with me through thick and thin.

Now I extend my thanks to my parents who have always been there for me right from the start.

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Date : /01/2004

Dr. S. Arif Ali Subzwari

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INTRODUCTION

Introduction

Acute viral hepatitis is a major health hazard in both developed and developing countries. The six hepatotropic viruses A,B,C,D,E and G have been causing considerable morbidity and mortality especially in developing countries. As it is a well documented fact that HAV and HEV are feco-oral spread and ours being an over populous, poverty stricken country, with most of the people not having proper sanitation. These viruses have been causing myriad, voluminous cases of hepatitis that are mostly subclinical and recover, and may never even come to the clinician. But there is a sizeable proportion of those in whom the various complications of acute viral hepatitis i.e., hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, hemorrhagic manifestations, chronic hepatitis, fulminant hepatic failure, hepatocellular carcinoma occur. These are the children who are in dire need of hospitalization.

On account of the high cost and unavailability of commercial kits routinely, there is dearth of literature, with regards to acute viral hepatitis in Bundelkhand. The main queries that we have tried to solve in our study are – The age related distribution of children afflicted with acute viral hepatitis along with its complications, the pattern of the biochemical parameters, which is serum bilirubin, SGPT, PT, and their

statistical significance and lastly testing the sera of these children by commercially available kits for the four hepatitides A, B, C, and E.

The exact incidence of Hep. A is not known on account of a high proportion of asymptomatic cases. Hep. B is a global health problem with > 2 million affected world wide and 350 million carriers. In India 6 % of the population is affected. Hep. C virus is infecting 3% of the world at present. In India exact incidence is unknown because of asymptomatic cases. 2 – 5 % of the blood donors are positive for HCV in India. In India > 50% acute viral .H is caused by non A / non B/ non C enterically transmitted virus which is HEV. It occurs in all epidemic , endemic , sporadic forms in India. It has a very high mortality rate because of fulminant liver failure in pregnant females in South East Asian Region (20%).

Hep. A , E are spread by faecal contamination of water while Hep. B is spread by bld. / bld. products and contact with body fluids / sexual contact. HCV is similarly transmitted parenterally. HGV is limitedly similar to HCV.

HBV is DNA virus while HCV, HAV, HEV & HGV are RNA viruses. For the purpose of diag. Of these viruses serological tests have been the main stay to this day on account of being able to confirm / document even asymptomatic cases.

HAV / HEV is best diagnosed by IgM antibody (detected by ELISA) presence that appears within 1-2 wks. and persists for 3-4 m. ELISA has the highest sensitivity(99.9%) and specificity(99.9%) so this test will be one of the tools of the study.

HBV has three kinds of antigens HBsAg, HBcAg and HBeAg. HBeAg denotes infectivity of the infected person while HBcAg does'nt appear in bld. and remain in the liver. HBsAg is the first serological marker to appear in the serum after 1-2 wks. and disappears in 1-2 m following onset of jaundice. Anti HBcAg IgM is a marker of acute infection apart from HBsAg and comes after 1-2 wks. of HBsAg appearance.

Though there is no dearth of literature on acute viral hepatitis and its complications in the Pediatric population in the rest of the country. Yet, Bundelkhand stands apart in this aspect.

On account of the cost effectiveness and early and protracted course of HBsAg this will be employed as the marker of choice for HBV detected by **LATEX. Agglutination method** (sensitivity 99% specificity 99%) though ELISA (sens. 100% sp. 99.9%) is superior.

HCV detection is based on anti-HCV antibodies that appear after 3-11 wks. of infection. Persistence is variable. Two types of tests are available ELISA and RIBA (Recombinant Immunoblot Assay). RIBA

has a sen. /sp. 100% and is superior to ELISA therefore this will be the fourth tool of the study.

Our main aim was to segregate and study children suffering from acute viral hepatitis who were hospitalized on account of its various complications, on the basis of history, clinical findings and liver function tests(S. Bilirubin, SGPT & PT) and who have been coming to the medical college in sizeable chunks year after year signifying the high morbidity and mortality on account of acute viral hepatitis and its complications in this region.

The main queries that we have tried to solve in our study were the age related distribution of children afflicted with acute viral hepatitis along with its complications, the pattern of the biochemical parameters which is serum bilirubin, SGPT, PT and their statistical significance and lastly testing the sera of these children by commercially available kits for the four hepatitides A,B,C & E.

AIMS AND OBJECTIVES

Aims & Objectives

- 1) To study the etiology in children admitted with complicated acute viral hepatitis in pediatric age group.
- 2) To study the age/sex related distribution of the hepatitide in children admitted with complicated acute viral hepatitis in pediatric age group.
- 3) To study the clinical features, complications and associated mortality in the various types of hepatitis in pediatric age group.
- 4) To study the biochemical parameters serum bilirubin, SGPT and prothrombin time and their statistical significance in different types of complicated viral hepatitis in children.

*REVIEW OF
LITERATURE*

Review of literature

Although hepatitis was known very early as an infectious disease (Zukermann 1979), the observations leading to our current knowledge of hepatitis began with studies of hepatitis outbreaks during the first world war and the demonstration later by transmission studies with bacteria free filtrates that the disease was caused by a virus. Human volunteer studies distinguished between faeco-oral transmitted, short incubation, epidemic (but also sporadic) hepatitis (hepatitis A) and parenterally transmitted long incubation hepatitis (hepatitis B) and indicated existence of further forms of hepatitis nonA, nonB. The search commencing with hepatitis B in 1963 leading to hepatitis E in 1990 terminated to the present day characterization of the six hepatotropic agents (A,B,C,D,E,G) (Shleizeinger).

All forms of viral hepatitis have a basic pathology. The essential lesion is an acute inflammation of the entire liver. Hepatic cell necrosis is associated with leucocytic and histiocytic reaction and infiltration. (Dible et al 1943).

All of them start with a prodromal period of mild fever, headache, flu like illness or no symptoms at all followed by darkening of urine/ lighting of faeces. Then jaundice develops along with

transient itching. Liver is specially affected and is palpable with a smooth/ tender edge in 70%. After an icteric period of 1-4 weeks the child gradually recovers. Hepatitis A,E and G are such type of self limiting illnesses. On the other hand hepatitis B and C are prone to chronicity.

Tandon et al (1984) studied etiological spectrum of viral hepatitis and the prevalence of serological markers of hepatitis A and B virus infection in healthy persons in North India. Hepatitis A virus was found to be the most common cause of acute hepatitis in children (67%). It was a less frequent cause of this disease in adults (14%). Exposure to HAV occurs in early etiological and by the age of 10 years 90% of healthy persons have serological evidence of hepatitis A virus infection.

Worldwide, there are more than 350 million carriers of HBV, 60 million of whom will die from liver cancer and 45 million from cirrhosis (Shleizeinger). The world wide prevalence of HBV infection is falling (Alberti et al).

It is estimated that there are 300 million carriers of HCV, about 2.5 million in Europe. In USA, it is conservatively estimated that approximately 170 000 cases of acute hepatitis C occur per year. Of

these, between 70 and 80% will maintain infection and develop chronic hepatitis (Alter et al).

Hepatitis E occurs in all epidemic, sporadic, endemic in India. The largest epidemic in India was reported in 1955-56 in New Delhi. Subsequent outbreaks of epidemic and sporadic hepatitis E were documented in North India (Tandon et al, 1982) and in Kashmir, India (Khuroo et al, 1983). Similar outbreaks have been reported in other regions of South East Asia. A high mortality rate (18-20%) has been incurred in pregnant women.

On studying the age related distribution of children with acute viral hepatitis (with complications). We found that various pediatricians.

Malathi et al (1998) Tondon et al (1999), Thapa et al (1995), Khuroo et al (1983) and many other workers have opined less than 10 years as the bench mark for enterically transmitted hepatitis with peak incidence of HAV in less than 5 year age group.

Malathi et al (1998) also subdivided 127 children with acute viral hepatitis into two years slabs (0-2, 2-4 and so on upto 12 years) they observed maximum incidence 43% of HAV between 2-4 years, 4-

6 years. Majority of Non A and Non B (they didn't test for HEV and HCV) were between 2-4 years.

Khuroo et al (1983) observed that HAV cases were maximum below 10 years of age.

Thapa et al (1995) subdivided their 324 children of acute viral hepatitis into three broad groups (0-5, 6-10 and 11-15 years) they found HAV in 50% of cases that were below 5 years followed by 36% in between 6-10 years.

In less than 10% patient extra hepatic immune complex mediated manifestations may be present like polyarthritits, (which is typically symmetric involves chiefly the distal joints e.g. proximal interphalangeal joints and subsides with development of jaundice), hematuria and proteinuria relecting glomerular involvement (Lister-Melman et al 1989), angioedema, urticaria, maculopular rash, polymyalgia rheumatica, neuropathies, myocarditis etc. (Bacon et al, 1975, Tabor 1987, Ussell et al 1984).

Thappa et al 1961, Matheisen et al 1979, Khuroo et al 1983, Matathi et al 1988 found male preponderance in children afflicted with acute viral hepatitis.

Stewart et al (1978), Lemon (1985) reported increased incidence of diarrhoea and vomiting in children with HAV. In a study on 415 patients with hepatitis A, Gust and Feinstone (1988), observed anorexia in 90% cases, Nausea in 87% cases, vomiting in 71% cases, abdominal discomfort in 65% cases, dark coloured urine in 94% cases and fever in 75% cases.

On examination of a case of acute viral hepatitis with or without complication lymphadenopathy, oedema, ascites, splenomegaly are found with varying incidence. Splenomegaly is found in 15% cases of acute viral hepatitis (Shleizeinger).

Fulminant hepatitis is marked by clinical features of hepatic synthetic function with associated bleeding diathesis and coma.

The myriad complications of acute viral hepatitis include bleeding manifestation presenting in the form of hematemesis / hemorrhagic RTA, malena, ascites, chronic hepatitis, fulminant hepatic failure, spontaneous bacterial peritonitis, hepatic encephalopathy and lastly acute renal shut down. While community studies of acute viral hepatitis have reported lower incidence of these complications on account of a high proportion/ chunk of self limited/ mild cases the studies on hospitalize children documented higher incidence.

Likewise amongst the community studies Malathi et al (1998), in their study on 127 children with acute viral hepatitis observed HE in 10.2% of cases with HAV, 17.6% cases with HBV and in 33.35 cases with combined A and B hepatitis.

Khuroo et al (1983), in their study on 293 cases with acute viral hepatitis observed that the incidence of HE in Non A and Non B hepatitis group (12.3%) was higher than that in hepatitis B group (4.2%) and hepatitis A group (6.8%).

Tandon et al (1985), and Kar et al (1994) in their study observed hepatic encephalopathy in maximum cases (55%) with Non A and Non B hepatitis.

Coming to studies on those children who were admitted on account of the complications of acute viral hepatitis. Poddar et al (2002) in their 172 children of acute viral hepatitis found ascites in (30%), encephalopathy in 32.6%. 16% children with ascites had spontaneous bacterial peritonitis.

Poddar et al (2000) again studied fulminant hepatic failure in 67% children they found ascites in 34% out of which 26% had spontaneous bacterial peritonitis.

Poddar et al (2000) and again Poddar et al (2002) in both their studies on acute viral hepatitis and fulminant hepatic failure respectively found that mortality was higher in those with spontaneous bacterial peritonitis occurrence.

BIOCHEMICAL PARAMETERS

Khuroo et al (1983), studied serum bilirubin levels in 293 children with AVH, the observed mean serum bilirubin levels by them were $2.1 \pm 2.1 \text{ mg\%}$ in HAV, $5.1 \pm 4.3 \text{ mg\%}$ in HBV and $4.8 \pm 4.5 \text{ mg\%}$ in NonA NonB hepatitis respectively. They found high levels of bilirubin in children infected with HBV, as compared to other viral markers.

Khuroo et al, observed that serum bilirubin levels in case of hepatitis A ($2.1 \pm 2.1 \text{ mg\%}$) were significantly less when compared to HBV and Non A and Non B hepatitis (p value < 0.05).

Mathiesen et al (1979) in their study on 115 patients in Copenhagen, also observed that the patients with type A hepatitis had a significantly lower levels of maximum bilirubin than those with type B (p value < 0.05).

Malathi et al (1998), studied serum bilirubin levels in 127 children with AVH, the observed mean serum bilirubin levels by them were $3.4 \pm 2.6 \text{ mg\%}$ in HAV, $5.8 \pm 2.2 \text{ mg\%}$ in HBV, $9.1 \pm 1.4 \text{ mg\%}$ in A

and B co-infection, and $5.8 \pm 2.5 \text{ mg\%}$ in Non A NonB hepatitis. No statistical significant inference was drawn by them.

SGPT

Mathiesen et al (1979), in their study on 115 patients found no statistical difference in maximum ALT levels of A,B and Non B hepatitis.

Mean ALT levels observed by Malathi et al (1998) were 227.1 ± 261.4 IU/ml in HAV, 360.8 ± 341.8 IU/ml in HBV, 741.3 ± 248.6 IU/ml in A+B co-infection and 407.3 ± 318.7 IU/ml in Non A NonB hepatitis. No statistical inference was drawn by them. Higher levels of ALT were observed in children with type B hepatitis alone or in combination with hepatitis A. by Mathiesen et al (1979 and Malathi et al (1998).

Prothrombin time It reflects the synthetic function of the liver and being protein with short half-life as compared to albumin. It is a good indicator of liver injury in acute viral hepatitis.

Fulminant hepatitis is marked by clinical features of hepatic synthetic function with associated bleeding diathesis and coma and PT is elevated.

Poddar et al (2000) graded PT as the marker of disease activity and the best indicator of prognosis in acute viral hepatitis with

complications. Fulminant hepatic failure was associated with decreased SGPT and increased prothrombin time (Poddar et al 2002).

In their study in hospitalized children with fulminant hepatic failure they found that PT was elevated significantly in those children who died than those who recovered.

Serological tests :-: For the purpose of diagnosis of these viruses serological tests have been the main stay to this day on account of being able to confirm/document even asymptomatic cases.

In an attempt to overcome the limited sensitivity of IEM assay have been developed for the detection of specific viral antigens and HAV-RNA. A number of workers (Hollinger et al 1975, Purcell et al 1976) developed sensitive immuno assay, radioimmuno assays (RIA) or ELISA for detection of HAAq in fecal samples.

HAV/HEV is best diagnosed by IgM antibody (detected by ELISA) presence that appears within 1-2 weeks and persists for 3-4 m ELISA has the highest sensitivity (99.9%) and specificity (99.9%).

Jansen et al (1985) detected HAV-RNA in fecal samples from patients with hepatitis A by molecular hybridization. This technique has been found to be more sensitive than ELISA or RIA for detection of HAAq.

Jiang et al (1987) described improved method of detection of HAV RNA by dot blot hybridization involving ^{32}P labeled single standard probes.

HBV has three kinds of antigens HbsAg, HbcAg and HbeAg, HbeAg denotes infectivity of the infected person while HbcAg doesn't appear in blood and remain in the liver. HbsAg is the first serological marker to appear in the serum after 1-2 weeks and disappears in 1-2 m following onset of jaundice. Anti HbcAg IgM is a marker of acute infection apart from HbsAg and comes after 1-2 weeks of HbsAg appearance.

Australian antigen of HbsAg could be detected inpatients with acute and chronic disease by simple assay procedures such as agar gel diffusion (AGD) or counterimmunoelectrophoresis (Gerety et al, 1978).

In 1972, a modified radioimmunoassay (RIA) called "Sandwich" RIA was developed by Overby et al, to detect HbsAg. This diagnostic test has a sensitivity 10000 times that of AGD and can detect less than 0.5ng HbsAg per ml of serum. Sandwich assays have remained the methodologies of choice for detecting HbsAg because of their long history of high sensitivity and specificity. Recent modified IEA have

employed microparticles (MEIAs) and computerized instrumentation to produce very rapid and completely automatic MIAS for HbsAg (Decker 1991, Eble et al 1991).

Serological tests for HCV detect antibodies to viral antigens. The first generation ELISA test used recombinant antigen c100. subsequent tests have used HCV recombinant and synthetic peptides and these have proved more sensitive and specific. The third generation ELISA includes antigens from the putative core, NS3, NS4 and NS5 regions of the virus (Courouce et al, 1994). These have a sensitivity and specificity of 99%.

The original anti c100 appeared only 4-6 months and even up to 1 year after the infection, whereas the antibody to c33 appears early at 11 weeks and always within 20 weeks of the onset. False positives still occurs and the mean period between infection and detection of antibody is 12 weeks (Busch et al, 1994). ELISA blood donor screening is virtually 100% effective in preventing transmission of HCV to recipients (Van der Poel et al, 1994).

MATERIAL & METHODS

Material & Methods

The study was conducted in the Department of Pediatrics / Microbiology M.L.B. Medical College Hospital, Jhansi.

Selection Of Cases

Our study comprised of 50 children, 0 – 18 years who were admitted from the OPD, emergency and in the Pediatrics ward on account of one or the other complications of acute viral hepatitis, over a period of one year were subjected to –

- a) Detailed fever and jaundice regarding onset and disappearance, duration of fever.
- b) Questions regarding extra hepatic manifestations (arthritis, urticaria, pancreatitis etc.) were asked and full physical examination conducted for the same as well as for the myriad complications of acute viral hepatitis, that made these children to present in the Medical College.
- c) Serological samples for elevation of liver enzymes (SGOT, SGPT), serum bilirubin was withdrawn for confirmation of liver involvement and particular importance was given to prothrombin (PT) time.

Biochemical parameters Serum bilirubin, SGPT and Prothrombin time were analyzed for each patient. Lastly, the four hepatitides were tested by analyzing the sera of these children.

Sera of these children was stored at $2 - 8^{\circ}\text{C}$ for upto 48 hours and frozen at 20°C or lower for longer periods as and when required. Repeated freeze thawing was avoided.

Patient sample was diluted 1 – 101 prior to use.

TEST FOR HEPATITIS A / E VIRUSES (By ELISA Kit Method)

The cloning and sequencing of HEV / HAV genome has led to the development of serological tests for detection of anti HAV IgM and anti HEV IgM antibodies.

Content of the kit

Microplate valves are coated with the virus specific immunodominant synthetic antigens. In the first incubation, the antibodies present in the sample bind the solid phase antigens. The bound anti HEV IgM, if any, are than detected during a second incubation by an enzyme conjugate that generates an optical signal that is proportional to the amount of antibodies present in the sample.

Contents of the kit

- 1) Microplates
- 2) Conjugate
- 3) Negative control

- 4) Positive control
- 5) Washing buffer
- 6) Substrate
- 7) Stop solution
- 8) Sample diluent
- 9) Micro pipettes of 10, 100 and 1000 μ l
- 10) Vortex mixture and adsorbent paper
- 11) ELISA grade water for dilutions
- 12) Timer
- 13) Photometric ELISA reader
- 14) Incubator set at 37°C.
- 15) Automated microplate washer

Essay procedure

All reagents and specimen were allowed to come to room temperature before use. All reagents were mixed without foaming.

Once started all steps were completed without interruption.

New disposable pipettes were used for each reagent.

Steps

- 1) dispense ready to use controls and diluted samples into wells.

Position	Calibrator/sample
A1	Blanking well
B1+ C1	100 μ l of negative control
D1	100 μ l of positive control
E1 H12	100 μ l of samples

- 2) Strips were incubated for 60 min at 37°C.
- 3) Microplate was washed .
- 4) In the mean time conjugate was prepared.
- 5) 100µl diluted conjugate was added.
- 6) Microplate was again washed.
- 7) 100µl of substrate was added, then incubated for 20 mins protected from light.
- 8) Enzymatic reaction was stopped by adding 100ml of stop solution.
- 9) Microplate was read at 450nm and 620-630 nm within 60mins. Blanking the instrument on A₁ well.

Calculation of results

Cutoff value was calculated by the formula

$$NC+0.250 = \text{cutoff}$$

Samples with an OD 450nm value lower than the cut off were classified as negative for HEV/ HEV IgM.

Samples with an OD 450nm value higher than the cutoff were classified positive for anti HEV IgM/ anti HAV IgM.

TEST FOR HEPATITIS B (HBsAg, By LATEX agglutination method)

Kit provided Tulip (Virutex HBsAg)

Principle- LATEX particles coated with anti HBsAg antibodies will agglutinate when mixed with serum or plasma containing HBsAg.

Reagent: It is ready to use suspension of polystyrene latex particles. These particles are coated with IgG class of monoclonal anti HbsAg antibodies.

Contents on the kit

- 1) Reagent pack
- 2) Having positive and negative control
- 3) Accessories pack having glass slide with 6 reaction circles mixing sticks, rubber teats, pipettes.

Procedure :

- 1) One drop of sample was pipetted on to one of the reaction circles of the glass slide.
- 2) Samples of 1:40 dilution (0.05ml serum + 1.95ml isotonic saline) were prepared.
- 3) One drop of diluted solution in the next reaction circle of glass slide.
- 4) One drop of positive and negative control was placed on to the remaining circles.
- 5) One drop of latex reagent was added to each of the samples and controls on the slide.
- 6) Mixing was done.

- 7) Stop watch was started.
- 8) Observing for agglutination at 5 minutes.

Interpretation of results

- 1) No agglutination with diluted and neat samples was a negative test result.
- 2) Agglutination with neat sample but no agglutination with diluted samples was a positive test result.
- 3) Agglutination with diluted sample and neat sample both was a positive.
- 4) Agglutination with diluted sample but not with neat was a positive.

TEST FOR HEPATITIS C (By RIBA, Recombinant Immunoblot Assay)

Kit By – Signal

Principle

Recombinant antigens (Ns3, Ns4, Ns5 & Core) of HCV are spotted on to the membrane of the test device. Antibodies present in the test serum react with these antigens and attach to the solid phase. Non-reactive antibodies are filtered through the wash buffer. HCV antibodies are visualized by reacting with COLLOIDAL GOLD PROTIEN A – Signal reagent. Appearance of two magenta red spots /

dots (one for the control and other of HCV antigens) indicate positive reaction.

Reagent / accessories

Reagent 1 : Test device (membrane with recombinant HCV antigens and control spot.

Reagent 2 : Wash buffer

Reagent 3 : Signal reagent

Accessories : Plastic dropper

Essay procedure :

- 1) Add 2 drops of wash buffer were added to the center of the test device and allowed to soak in.
- 2) Two drops of patients sample (100 μ l) were added.
- 3) Two drops of wash buffer again.
- 4) Two drops of signal reagent.
- 5) Two drops of wash buffer for the third time.
- 6) The results was read after 10 mins.

Interpretation

Positive results if two red magenta dots /spots.

Negative results if only one red magenta dot / spot (for control).

Invalid test if no dot / spot appears.

OBSERVATIONS

Observations

The present study was conducted in the department of Pediatrics/ microbiology of M.L.B. Medical College, over a period of one year on children 0 to 18 years, admitted from the Out Patient Department, in the emergency and the pediatrics ward of M.L.B. Medical College, Jhansi.

Our study group comprised of 50 children from 0 to 18 years who were admitted on account of one or the other complications of acute viral hepatitis. The diagnosis of acute hepatitis was made on the basis of history, clinical findings & biochemical investigations such as S. Bilirubin, ALT, and AST. Prothrombin time was used as the most important criteria to document the severity of hepatitis and also used along with SGPT during the follow up of the cases, as marker of disease activity. Sera of these children was analyzed for viral markers anti HAVIgM, anti-HEV using ELISA Kits from EIAGEM HAVIgM, HEVIgM for diagnosis of HAV and HEV & HbsAg by Latex agglutination method diagnosis for HBV and HCV by immunoblot assay kit by Signal.

Detailed history regarding the onset of fever, flu like illness, jaundice, their duration, subsidence, colour of urine, lightening of feces was inquired into. History regarding extrahepatic manifestations such as rash, urticaria renal involvement, seizures was also taken.

Patients were then subjected to detailed physical examination with special emphasis on rashes, purpura, petechae and icterus.

Liver span and spleen span were noted to look for hepatomegaly and splenomegaly along with shifting dullness and fluid thrill for ascites, guarding and rigidity for peritonitis if present.

Level of consciousness along with examination of reflexes, planters, and detailed CNS examination was conducted for grading of hepatic encephalopathy (I,II,III and IV).

TABLE 1A

Age in (yrs)	Total no. of cases (n = 50)	Percentage
0-2	4	8
2-4	7	14
4-6	11	22
6-8	9	18
8-10	8	16
10-12	4	8
12-14	3	6
14-16	3	6
16 – 18	1	2

It was observed that out of 50 children presented with complicated acute viral hepatitis, majority of cases 11 (22%) were between the age of 4-6 years followed by 9 (18%) children between 6-8 years of age. Least number of cases 1 (2%) was present between 16 – 18 years of age.

TABLE 1B

Age in years	HAV	HBV	HCV	HEV
0-2	2	1	0	1
2-4	3	1	0	2
4-6	8	0	0	3
6-8	6	0	0	3
8-10	4	1	0	1
10-12	1	1	0	1
12-14	1	1	0	1
14-16	0	2	1	0
16-18	0	1	0	0
Total	25	8	1	12

The age related data in our 50 children with complicated acute viral hepatitis is depicted in table 1C. The maximum no. of cases in HAV have clustered before ten years especially before 5 years. Similarly maximum no. of cases of HEV have clustered before ten years.

TABLE 2

Sex	Total
Boys	30 (60 %)
Girls	20 (40 %)
Total	50

Table 2 shows the sex related data for complicated acute viral hepatitis, being 60% for boys and 40% for girls. This shows that there is male preponderance and the ratio of boys : girls being 3 : 2.

TABLE 3A

Sex	HAV	%	HBV	%	HCV	%	HEV	%	Total
Boys	15	30	5	10	1	2	8	16	29
Girls	10	20	3	6	0	0	4	8	17
Total	25		8		1		12		46

Table 3 reveals the sex related data of complicated acute viral hepatitis, in the respective group of hepatitides. This table shows male preponderance in all the groups of hepatitides with HAV the ratio being 3 : 2, HBV ratio 5 : 3, in HEV being 2 : 1. Boys were slightly highly infected than girls by all types of hepatitides.

TABLE 3B

Complications	No. of cases	Percentage
Haematemesis / hemorrhagic RTA	15	30
Malena	15	30
Ascites	11	
Hepatic encephalopathy	19	38
Chronic hepatitis	1	2
Fulminant hepatic failure	8	16
Spontaneous Bacterial peritonitis	2	4
ARF	5	10

Table 3B depicts the overall incidence of complications in our children of complicated acute viral hepatitis.

TABLE 4

Symptoms	HAV	%	HBV	%	HCV	%	HEV	%
Anorexia	20	40	4	8	0	0	6	12
Nausea	15	30	4	8	0	0	4	8
Vomiting	15	30	4	8	0	0	6	12
Fever	20	40	6	12	1	2	10	20
Jaundice	17	34	8	16	1	2	6	12
Dark Urine	20	40	6	12	0	0	6	12
Pruritis	10	20	4	8	0	0	2	4
Rashes	4	8	1	2	0	0	0	0
Altered Sensorium (encephalopathy)	10	20	4	8	0	0	5	10
Seizures	10	20	4	8	0	0	5	10
Anicteric	8	16	-	-	-	0	-	-
Abdominal discomfort	20	40	6	12	0	0	6	12

Out of 50 children presenting with complicated acute viral hepatitis, gastrointestinal manifestations such as anorexia (40%), nausea (30%), vomiting (30%) and abdominal discomfort (40%) were more common in children affected with HAV than the hepatitis caused by other viruses. Fever (40%) was also more common in type A hepatitis as compared to hepatitis caused by other viruses. Dark coloured urine was present in children (40%) affected by HAV, 12% in HBV, nil in HCV and 12% in HAV. Jaundice was present in 34%

HAV cases, 16% of HBV cases and 2% of HCV cases and 12% of HEV cases. Anicteric hepatitis was seen in 16% of HAV cases only.

Pruritis was present in 20% cases of HAV, 8% of HBV, nil cases of HCV and 4% cases of HEV. Rashes were present only in the cases of HAV and HBV only as 8% and 2% respectively. Altered sensorium was more common HAV cases (20%), followed by HEV (10%) and HBV (8%).

TABLE 5

Signs	HAV	%	HBV	%	HCV	%	HEV	%
Anemia	4	8	2	4	1	2	0	0
Hepatomegaly	12	24	4	8	1	2	4	8
Hepatosplenomegaly	8	16	4	8	0	0	5	10
Lymphadenopathy	6	12	4	8	0	0	2	4
Oedema	15	30	4	8	0	0	4	8

On examination, it was observed that anemia was present in 8% of HAV cases, 4% of HBV cases, 2% of HCV cases and no case of HEV had anemia.

Liver enlargement was present in 24% cases of HAV being the most common hepatitis as far as hepatomegaly is concerned. 8% of HBV cases, 2% of HCV cases and 8% cases of HEV had hepatomegaly.

Liver enlargement along with splenomegaly was observed in 16% of HAV cases, 8% of HBV cases and 10% of HEV cases.

Lymph node enlargement though rare was observed in 12% of HAV, 8% of HBV children and 4% of HEV cases in our study.

Oedema was observed in 30% children affected with HAV, and 8% of both affected by HBV and HEV.

TABLE 6

Complications	HAV	%	HBV	%	HCV	%	HEV	%
Haematamesis / hemorrhagic RTA	10	20	2	4	0	0	3	6
Malena	10	20	2	4	0	0	3	6
Ascites	8	16	0	0	0	0	3	6
Hepatic encephalopathy	10	20	4	8	0	0	5	10
Chronic hepatitis	0	0	1	2	0	0	0	0
Fulminant hepatic failure	4	8	2	4	0	0	2	4
Spontaneous Bacterial peritonitis	2	4	0	0	0	0	0	0
ARF	3	6	1	2	0	0	1	2

Among 50 children presenting with complicated acute viral hepatitis complications like Gastrointestinal bleeding in the form of

haematemesis, hemorrhagic RTA or malena were mostly observed in 20% children affected with HAV, which was most common, 4% of HBV and 6% of HEV cases.

Ascites was present in 16% of HAV cases and 6% of HEV cases only. HAV being the commonest virus responsible for ascites.

Hepatic encephalopathy along with seizures, extensor planters and altered sensorium were most common with hepatitis A. In 20% of the cases (i.e. 10 out of 25), 8% of HBV cases and 10% of HEV cases presented with hepatic encephalopathy.

Chronic hepatitis was observed in a single patients of hepatitis HBV. Fulminant hepatic failure being the worst prognostic factor was associated most commonly with HAV (8%), followed by HBV and HEV 4% each respectively.

Spontaneous bacterial peritonitis, the most dreaded complication was observed in 2 cases of HAV (4%).

Acute renal failure was seen in 6% of HAV cases, 2% HBV and 2% of HEV cases.

TABLE 7

HE	HAV	%	HBV	%	HCV	%	HEV	%
Grade I	1	2	0	0	0	0	1	2
Grade II	1	2	1	2	0	0	1	2
Grade III	2	4	2	4	0	0	1	2
Grade IV	6	12	1	2	0	0	2	4
Total	10		4		0		5	

Table 7 depicts hepatic encephalopathy grades in the four types of hepatitis. HAV showed the maximum number of patients with encephalopathy i.e. 10 (20%), followed by hepatitis B virus 4 (8%) and HEV 5 (10%). Thus, the maximum number of patients with hepatic encephalopathy were those with HAV, being grade I 2%, grade II 2%, grade III 4%, grade IV 12%.

TABLE 8***CLINICAL OUTCOME OF AVH CASES***

	No of cases	Recovery	Death
A	25	19	6
B	8	6	2
C	1	1	0
E	12	11	1
Negative for viral markers	4	3	1
Total	50	40	10

Out of 50 cases of complicated acute viral hepatitis that we studied, we found that 12% died in HAV, 4% died in HBV, nil in HCV and 2% in HEV. Overall mortality in case of complicated acute viral hepatitis was 20%.

TABLE 9

	HAV	HBV	HCV	HEV	Negative for viral markers
Fulminant hepatic failure	2	0	0	1	0
Encephalopathy	1	2	0	0	1
Spontaneous bacterial peritonitis + fulminant hepatic failure	1	0	0	0	0
Spontaneous bacterial peritonitis	2	0	0	0	0

Table 9 depicts the complications leading to mortality in complicated acute viral hepatitis according to respective hepatitides.

The maximum no. of fulminant hepatic failure cases were observed in HAV (2). One case was positive for both fulminant hepatic failure and SBP in HAV and two cases of HAV had only spontaneous bacterial peritonitis.

TABLE 10

S. Bil (mg%)	HAV	HBV	HCV	HEV
2-5	10	2	0	4
5-10	4	2	0	5
10-15	5	2	0	2
15-20	4	1	1	1
≥20	2	1	0	0

This table shows the serum bilirubin levels in various group of hepatitides.

TABLE 11

SGPT/ALT	HAV	HBV	HCV	HEV
<100	4	2	0	2
101-500	6	2	0	2
501-1000	10	2	1	2
1001-1500	4	2	0	6
1501-2000	1	0	0	0
2001-2500	0	0	0	0
≥2500	0	0	0	0

This table shows the SGPT levels in the various group of hepatitides. Amongst the 50 children with complicated acute viral hepatitis that we have studied.

TABLE 12

Viral Hepatitis	Total No. Of Cases	Percentage
HAV	25	50
HBV	8	16
HCV	1	2
HEV	12	24
Negative for viral markers (drug induced, malaria, etc)	4	8
Total	50	100

Out of 50 children with complicated acute viral hepatitis, viral markers were positive in 92% cases, and 8% (4 out of 50) cases tested

negative for all the viral markers. HAV was the sole infecting agent in 25 (50%) cases, HBV in 8 (16%%), HCV in 1 (2%) and HEV in 12 (24%).

DISCUSSION

Discussion

Acute viral hepatitis is major health hazard in both developed and developing countries. The six hepatotropic viruses A,B,C,D,E & G have been causing considerable morbidity and mortality specially in developing countries. As it is a well documented fact that HAV and HEV are faeco oral spread and ours being an over populous, poverty stricken country, with most of the people not having proper sanitation. These viruses have been causing myriad, voluminous cases of hepatitis that are mostly sub clinical and recover and may never even come to the clinician. But there is a sizeable proportion of those in whom the various complications of acute viral hepatitis i.e. hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, haemorrhagic manifestations, chronic hepatitis, fulminant hepatic failure, hepatocellular carcinoma occur. These are the children who are in dire need of hospitalization.

Though there is no dearth of literature on acute viral hepatitis and its complication in the pediatric population in the rest of the country, yet bundelkhand stands a part in this aspect. On account of the high cost and unavailability of commercial kits routinely, there is dearth of literature with regards to acute viral hepatitis in Bundelkhand.

Our study comprised of 50 children (0-18 years) who were admitted from the OPD, emergency and in the pediatric ward on account of one or the other complications of acute viral hepatitis over a period of one year Dec 2002 to Dec 2003 in MLB Medical College, Jhansi.

On studying the age related distribution of children with acute viral hepatitis (with complications), we found that various pediatricians.

Malathi et al (1998) Tondon et al (1999), Thapa et al (1995), Khuroo et al (1983) and many other workers have opined less than 10 years as the bench mark for enterically transmitted hepatitis with peak incidence of HAV in less than 5 year age group. Our study also corroborates this fact as our maximum no. of HAV cases 11 (16%) were in the 4-6 year age group along with HEV cases that clustered maximum between 4-6 year and 6-8 year slabs.

Malathi et al (1998) also subdivided 127 children with acute viral hepatitis into two years slabs (0-2, 2-4 and so on upto 12 years) they observed maximum incidence 43% of HAV between 2-4 years, in contrast to 4-6 years in our study. Majority of Non A and Non B (they didn't test for HEV and HCV) were between 2-4 years. While our

HEV cases clustered between 4-6 years and 6-8 years. This difference can be attributable to the small sample size that we have taken.

Khuroo et al (1983) observed that HAV cases were maximum below 10 years of age with which our results are well in concordance.

Thapa et al (1995) subdivided their 324 children of acute viral hepatitis into three broad groups (0-5, 6-10 and 11-15 years) they found HAV in 50% of cases that were below 5 years followed by 36% in between 6-10 years which is comparable to our study as well. Thus it can be concluded that enterically transmitted hepatitis (HAV, HEV) is mostly acquired by 10 year of age and that incidence of HAV is more common before 5 year of age and HEV is more common in children less than 10 year of age.

So far male preponderance has been the order of the day as far as acute viral hepatitis in children is concerned. All the past workers Mathiesen et al 1979 (male : female ratio 1.4:1) Malathi et al 1998 (male female 2.1:1), Thapa et al 1961 (male : female 2:1) have found that.

This fact was reinforced by our study as well in which we found 30 cases (60%) to be boys and 20 cases (40%) to be girls out of a total of 50 (ratio male : female 1.5:1).

All types of viral hepatitis start with a prodromal period of mild fever, headache, flu like illness or no symptoms at all followed by darkening of urine/lightening of faeces. Then jaundice developed along with transient itching it is documented well in literature that gastro intestinal sign and symptom diarrhoea, anorexia, nausea, vomiting are more common in enterically transmitted agents (HAV,HEV).

We found that anorexia (40%), nausea (30%) & vomiting (30%), were more common with HAV and HEV than with other viruses. Fever was also more common in type A and type E hepatitis. Anicteric hepatitis was seen in HAV only (16%). Altered sensorium (20%) and seizures were more common in HAV.

Similar to our study, Stewart et al 1978), Lemon (1985) also reported increased incidence of diarrhoea and vomiting in children with HAV. In a study on 415 patients with hepatitis A, Gust and Feinstone (1988), observed anorexia in 90% cases, Nausea in 87% cases, vomiting in 71% cases, abdominal discomfort in 65% cases, dark coloured urine in 94% cases and fever in 75% cases. There findings are comparable with the present study. They observed altered sensorium in 49% cases of HAV which is in concordance with our study.

On examination of 50 cases of acute viral hepatitis (table-7), it was found that liver enlargement was present in 24% cases of HAV and HAV being the most common hepatitis as far as hepatomegaly was concerned. 8% of HBV cases, 2% of HCBV cases and 8% cases of HEV had hepatomegaly.

Liver enlargement along with splenomegaly was observed in 16% of HAV cases, 8% of HBV cases and 10% of HEV cases, which is corroborated by earlier literature that splenomegaly is present in 15% cases of acute viral hepatitis (Nelson Text Book of Pediatrics).

Lymph node enlargement though rare was observed in 12% of HAV, 8% of HBV children and 4% of HEV cases in our study.

Oedema was observed in 30% children affected with HAV and 8% of both affected by HBV and HEV.

One of the major aims of our study was to concentrate on the complications of acute viral hepatitis, which is what made these children to hospitalize and which are the main cause of increase mortality in acute viral hepatitis.

The myriad complications of acute viral hepatitis include bleeding manifestation presenting in the form of hematemesis / hemorrhagic RTA, malena, ascites, chronic hepatitis, fulminant hepatic

failure, spontaneous bacterial peritonitis, hepatic encephalopathy and lastly acute renal shut down. While community studies of acute viral hepatitis have reported lower incidence of these complications on account of a high proportion/ chunk of self limited/ mild cases the studies on hospitalized children documented higher incidence.

Coming to studies on those children who were admitted on account of the complications of acute viral hepatitis. Poddar et al (2002) in their 172 children of acute viral hepatitis found ascites in (30%), encephalopathy in 32.6%. 16% children with ascites had spontaneous bacterial peritonitis which is comparable to our results where we have seen ascites in 22%, encephalopathy in 40% cases. Spontaneous bacterial peritonitis occurred in 20% cases of ascites (2 cases out of 11) in our scenario.

Poddar et al (2000) again studied fulminant hepatic failure in 67% children they found ascites in 34% out of which 26% had spontaneous bacterial peritonitis which is comparable to our study.

Amongst the community studies Malathi et al (1998), in their study on 127 children with acute viral hepatitis observed HE in 10.2% of cases with HAV, 17.6% cases with HBV and in 33.35 cases with combined A and B hepatitis.

Khuroo et al (1983), in their study on 293 cases with acute viral hepatitis observed that the incidence of HE in Non A and Non B hepatitis group (12.3%) was higher than that in hepatitis B group (4.2%) and hepatitis A group (6.8%).

Tandon et al (1985), and Kar et al (1994) in their study observed hepatic encephalopathy in maximum cases (55%) with Non A and Non B hepatitis.

The major stress these days in complicated acute viral hepatitis is on the fulminant hepatic failure and the immediate antecedent complications (ascites, spontaneous bacterial peritonitis, hepatic encephalopathy) leading to mortality in this entity.

Clinical outcome of our 50 children on follow up (table-9,10) revealed HAV as the most common cause of mortality with fulminant hepatic failure (4%) as the most common immediate antecedent complication leading to this mortality and occurrence of spontaneous bacterial peritonitis was associated with poor prognosis.

Poddar et al (2000) and again Poddar et al (2002) in both their studies on acute viral hepatitis and fulminant hepatic failure respectively found that mortality was higher in those with spontaneous bacterial peritonitis occurrence which is in accordance with our results.

They also found that higher grade of encephalopathy was associated with worse prognosis.

Now coming to the biochemical parameters that were elucidated during the course of complicated acute viral hepatitis children which is serum bilirubin, SGPT and prothrombin time.

Khuroo et al (1983), studied serum bilirubin levels in 293 children with AVH, the observed mean serum bilirubin levels by them were 2.1 ± 2.1 mg% in HAV, 5.1 ± 4.3 mg% in HBV and 4.8 ± 4.5 mg% in NonA NonB hepatitis respectively. They found high levels of bilirubin in children infected with HBV, as compared to other viral markers.

In our study among 25 cases of HAV mean serum bilirubin level was 7.6 ± 4.5 while in 6 cases of HBV mean serum bilirubin level was 10.11 ± 6.02 mg%. In HEV it was $4.35 \pm .82$ mg% and in HCV 6.2 ± 2 mg%, no statistical significance was observed (p value >0.05) on comparison.

Khuroo et al, observed that serum bilirubin levels in case of hepatitis A (2.1 ± 2.1 mg%) were significantly less when compared to HEV and HCV hepatitis (p value <0.05). They found high levels of bilirubin in children infected with HBV, as compared to other viral markers. Khuroo et al, observed that serum bilirubin levels in case of

hepatitis A (2.1 ± 2.1 mg%) were significantly less when compared to HBV and Non A and Non B hepatitis (p value <0.05). in contrast no statistical significance (p value >0.05) was observed in our study, on comparing serum bilirubin levels of HAV with HBV and HCV and HEV case.

Mathiesen et al (1979) in their study on 115 patients in Copenhagen, also observed that the patients with type A hepatitis had a significantly lower levels of maximum bilirubin than those with type B (p value <0.05).

Malathi et al (1998), studied serum bilirubin levels in 127 children with AVH, the observed mean serum bilirubin levels by them were 3.4 ± 2.6 mg% in HAV, 5.8 ± 2.2 mg% in HBV, 9.1 ± 1.4 mg% in A and B co-infection, and 5.8 ± 2.5 mg% in Non A NonB hepatitis. No statistical significant inference was drawn by them.

SGPT has been the liver enzyme of choice so far as far as its correlation with the degree of liver damage in different types of hepatitis is concerned. Though prothrombin time has been considered as the marker of choice for assessing the liver injury. Mathiesen et al (1979), in their study on 115 patients found no statistical difference in maximum ALT levels of A,B and Non B hepatitis.

Mean ALT levels observed by Malathi et al (1998) were 227.1 ± 261.4 IU/ml in HAV, 360.8 ± 341.8 IU/ml in HBV, 741.3 ± 248.6 IU/ml in A+B co-infection and 407.3 ± 318.7 IU/ml in Non A Non B hepatitis. No statistical inference was drawn by them. Higher levels of ALT were observed in children with type B hepatitis alone or in combination with hepatitis A. by Mathiesen et al (1979 and Malathi et al (1998). Mean ALT levels observed by Malathi et al (1998) were not statistically significant similar to our study.

SUMMARY

Summary

This study entitled seroepidemiology of hepatitis A,B,C,E among children of hepatitis in Bundelkhand was conducted on children admitted from the OPD, in the emergency and in the pediatrics ward of MLB Medical College, Jhansi with assistance from the department of Microbiology. The period of study was one year extending from Dec 2002 to Dec 2003.

A sizeable chunk of patients who are having the various complications (haemorrhage, encephalopathy, acute renal shutdown, ascites, spontaneous bacterial peritonitis) present in the medical college.

There is dearth of literature when it comes to studies on children with complicated acute viral hepatitis in Bundelkhand and considering the sizeable proportions in which these children come to the medical college. This study came in to inception.

Viral hepatitis is caused by six groups of hepatotropic viruses A,B,C,D,E & G. These viruses are now diagnosed mainly by serological methods by commercially available kits for ELISA (A,E) and latex agglutination (B) and recombinant immunoblot assay C.

A total of 50 children in the age group 0-18 years who were admitted with presumptive diagnosis of complicated acute viral hepatitis were subjected to detailed history, clinical examination and

by a serological test. SGPT, PT and serum bilirubin for confirmation of acute viral hepatitis along with its complications then the mortality pattern of these viruses was also studied on follow up of these patients.

We found that out of our 50 children of complicated acute viral hepatitis HAV emerged as the most common etiology with 25 cases (55%). HAV being the second most common with 12 cases (24%) and HBV and HCV being 8 (16%) and 1 (2%) respectively.

On studying the age related distribution of these cases we found that HAV was more common before 10 years age especially 5 years and HEV cases clustered in less than 10 years. The peak age group for HAV was between 4-6 years and for HEV between 6-10 years.

There was male preponderance ratio 1.5:1 male : female. When sex related data was computed for each children afflicted with complicated acute viral hepatitis.

On studying the clinical features of various hepatitises we found anorexia, nausea, vomiting to be more common in HAV.

On examination splenomegaly was found in 20% of cases along with varying degrees of lymphadenopathy, oedema, ascites.

Now coming to the complications of acute viral hepatitis we found HAV as the commonest etiology leading to fulminant hepatic failure and hepatic encephalopathy. We found hepatic encephalopathy

in 20% cases of HAV and overall incidence of encephalopathy was 20% in our 50 children of complicated acute viral hepatitis.

HEV was the second most common virus responsible for complications in these children.

Mortality was maximum with children afflicted with hepatitis A virus and that too with fulminant hepatic failure followed by encephalopathy.

The maximum mortality was in those patients who had occurrence of spontaneous bacterial peritonitis in children with fulminant hepatic failure.

The biochemical parameters SGPT, serum bilirubin were not significantly related to any of the hepatitides as far as liver injury was concerned.

Prothrombin time was more in patients who did not see the light of the day than in those who recovered.

CONCLUSION

Conclusion

1. Hepatitis A virus is the most common etiology in children suffering from complicated acute viral hepatitis in children in bundelkhand. Hepatitis B being the second most common hepatitide in these children.
2. Hepatitis A virus is more common, has maximum incidence before ten years of age in acute viral hepatitis in pediatric age group, especially before five years.
3. Hepatitis E virus is more common before ten years of age.
4. There is male preponderance in cases of acute viral hepatitis in children.
5. Anorexia, nausea and vomiting are more common with enteric ally transmitted agents (hepatitis A virus and hepatitis E virus) in acute viral hepatitis in children.
6. Hepatitis A virus is the most common cause of fulminant hepatic failure in children suffering from complicated acute viral hepatitis and HEV being the second most common cause.
7. Mortality in complicated acute viral hepatitis is maximum with fulminant hepatic failure and occurrence of spontaneous bacterial perintonitis, in this scenario, is associated with poor prognosis.

8. There is no significant correlation of SGPT, serum bilirubin amongst the various hepatitides (A,B,C & E) in children with complicated acute viral hepatitis.

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